SL 2002-149

SHORT COMMUNICATION

Acceleration Effects on Pupil Size with Control of Mental and Environmental Factors

BOB CHEUNG AND KEVIN HOFER

CHEUNG B, HOFER K. *Acceleration effects on pupil size with control of mental and environmental factors.* **Aviat Space Environ Med 2003; 74:669–74.**

Background: Using still photography and cinematography, early studies reported that pupils dilate immediately on exposure to acceleration and the dilation persists during sustained +G_z. Due to the lack of control for mental and environmental factors and poor sampling rate in early techniques, we re-examined the effects of increased acceleration on pupil size in the human centrifuge. ***Methods:*** Eight volunteers were dark adapted for 20 min by fixating on a 2.5 mm LED disk, 1 m away in otherwise complete darkness. Pupil diameters and horizontal and vertical eye movements were recorded binocularly in real time using an eye-tracking and pupillometry system. The recording sequence consisted of 1 min at +1 G_z, followed by transition to +3 G_z at 0.1 $G \cdot s^{-1}$, 2 min at sustained +3 G_z, return to +1 G_z at 1 $G \cdot s^{-1}$, and finally maintained at +1 G_z for another minute. During this sequence, the subject's heart rate was also monitored and the subject was instructed to subtract 2 from 1000 to maintain mental alertness. ***Results:*** All subjects showed a significant decrease in pupil diameter ($p < 0.05$) and increase in heart rate ($p = 0.02$) during +3 G_z and regained the baseline level on returning to +1 G_z. The maximum decrease in pupil diameter during sustained acceleration ranged from 0.6 to 3.3 mm. ***Conclusion:*** Our findings suggest that pupils constrict during hypergravity. It appears that the reflex regulation of pupil size is attributed to parasympathetic action through fibers originating in Edinger-Westphal's nucleus in the mesencephalon, which receives direct input from the otoliths.

Keywords: pupil size, acceleration, centrifuge, hypergravity.

DILATATION OF THE PUPILS during increased +G_z (positive, headward, eyeballs down) acceleration has been widely accepted and therefore not thought worthy of further investigation. Wilson and Canfield (14) studied the effects of increased acceleration on pupillary size on seven male volunteers who had previous experience as subjects in the human centrifuge. The subject's face was photographed with a flash at both high and low level of illumination at +1 G_z. A few minutes later, the subject's face was photographed again at +5 or +6 G_z at intervals of 1, 3, 5, 9 and 10 s after G-onset. Although no quantitative data was presented, the authors concluded that the pupils dilated immediately on exposure to acceleration. It was unclear whether pupil dilation persisted during the entire exposure to increased acceleration. The authors hypothesized that the increase in pupillary size was a response of the sympathetic nervous system to the onset of a strong stimulus (presumably increased +G_z). A decade later, using a television camera and 16-mm cinecamera, Duane et al. (1) reported that during increased acceleration, concomitant with the loss of peripheral vision, pupillary dilatation began and reached a maximum with the loss of central vision. It was proposed that cerebral hypoxia produced by positive acceleration was responsible for the pupillary dilation. Subsequently, the observation of pupillary dilatation was proposed as a useful quantitative sign for evaluation of physiological response to increased acceleration.

In later years, Oosterveld et al. (9) investigated the effect of otolithic stimulation on human pupillary size by exposing subjects to parabolic flight maneuver. The parabolic flight profile produced approximately 10 s of microgravity alternating with an increased G-load of +2 G_z during the pull-up phase. Continuous pupil observations of one eye were made with a video camera. In 65 out of a total of 68 subjects, dilatation of the pupil was observed immediately with the introduction of microgravity and conversely pupil constriction was observed in 55 cases during increased acceleration (+2 G_z). This finding appears to be at variance with earlier centrifuge studies (1,14) described previously.

Physiologically, the constant modulation of pupillary responses brought about by the spontaneous shifts in autonomic innervation is one reason why results of pupillary experiments are given only as a single observation, averages, or as percent changes over a preceding condition. Without the original data concerning pupillary size and control of eye movements, it is difficult to be sure of what actually happened. In this study, we employed a real time, noninvasive, objective measurement of pupil diameter using an infrared eye tracking and pupillometry technique to re-examine the effect of increased acceleration on pupil size.

From the Defence Research and Development Canada–Toronto, Ontario, Canada.

This manuscript was received for review in October 2002. It was revised in December 2002. It was accepted for publication in January 2003.

Address reprint requests to: Dr. Bob Cheung, Senior Defence Scientist and Project Leader for Disorientation Countermeasures and Vestibular Studies, Defence Research and Development Canada–Toronto, 1133 Sheppard Avenue West, P. O. Box 2000, Toronto, Ontario, Canada, M3M 3B9; E-mail: bob.cheung@drdc-rddc.gc.ca.

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METHODS

Subjects

Eight subjects (one woman and seven men) ranging in age from 23 to 42 yr participated in this study. Approval for this study was obtained from the DRDC-Toronto (formerly DCIEM) Human Ethics Committee. Subjects gave informed consent and completed a medical history questionnaire. All subjects had no known history of oculomotor, ophthalmologic, or vestibular disorder, and they had neither spontaneous nystagmus nor a Romberg sign in darkness. Spontaneous nystagmus is involuntary eye movements that are direction fixed and beat with about the same intensity in all head positions when the eyes are closed. Romberg sign is the inability to maintain stable posture when standing on the floor, feet in heel-to-toe position, with eyes closed, for 1 min. The appearance of spontaneous nystagmus and/or Romberg sign is considered to be a sign of vestibular pathology. The subjects were instructed to strictly abstain from alcohol, tobacco (cigarette smoking), other stimulants, and over the counter or prescribed medication for at least 24 h prior to testing. These substances might affect pupillary size, vestibular function, and potentially aggravate the disorientation experienced especially when returning from hypergravity to a +1 G_z baseline level. All the subjects that participated in the study were experienced centrifuge subjects.

Motion Stimulus

The experiment was conducted in the DRDC-Toronto human-rated centrifuge, which consists of a rotating arm 6.1 m long from the center of rotation to the pivoting point of the gondola. The gondola is passively coupled to the G_z vector and pivots on self-aligning trunnion bearings. The gondola swings out freely during rotation, such that the resultant vector of gravitational and centrifugal acceleration is always orthogonal to the floor of the gondola. Consequently, the subject inside the gondola experiences an increased downward-pulling force in the direction of their spinal (z) axis, thus achieving a +G_z centrifugation. In the present study, the resultant force either equaled Earth's gravity (+1 G_z; centrifuge stationary) or exceeded it by a factor of 3 (+3 G_z; centrifuge rotating at approximately 20 revolutions per minute). The seated subject was secured in the gondola facing the direction of motion of the centrifuge.

Eye Movements and Pupil Diameter Monitoring

Vertical and horizontal eye positions and pupil diameter of both eyes were monitored in real-time throughout each trial using the El-Mar video-based binocular eye-tracking system (Series 2000, El-Mar Inc., Toronto, Ontario). Vertical and horizontal eye positions were recorded to ensure that the subjects' eyes remain fixated on a LED target throughout the entire trial. The vertical eye position was also used to confirm eye blinks that might have affected pupil size measurement.

Heart Rate Monitoring

The subject was equipped with Marquette ECG Monitor, (Case 12 Model, Milwaukee, WI) as an indicator for +G_z stress. ECG was continuously monitored using five unipolar limb leads of V5 (left chest), LL (left leg), LA (left arm), RA (right arm), and RL (right leg) as an indication of +G_z stress. Heart rate was determined from the electrocardiograms because the time interval between two successive beats is the reciprocal of the heart rate. If the interval between two beats as determined from the time calibration lines is 1 s, the heart rate is 60 bpm.

Procedure

Each subject participated in one session approximately 5 min in duration, which included Pre, +3 G_z, and Post segments. The eye tracking assembly was mounted on a Canadian Forces standard flight helmet mechanically attached to the seat of the gondola. As a result, the subject's head is positioned so that the plane of the lateral semicircular canals was approximately parallel to the earth horizontal axis. The head restraint allowed a maximum of 2 cm vertical translation of the subject along the spine (z-axis). This was necessary, as increased acceleration tends to cause the subject to sink down into the seat. In addition, the head restraint prevents any movement about the roll, pitch, and yaw plane or any translation along the x- and y-axes.

After the eye tracking system was calibrated, the subjects were dark adapted for 20 min with only a red LED light (a 2.5 mm red LED disk) located 1 m away in front of the subject in otherwise complete darkness. During the trials, the subject was instructed to fixate on the LED target to control for vergence movements of the eyes and luminance. The run profile for the centrifuge is as follows: Pre-test at ground level (+1 G_z) was maintained for 60 s (Fig. 2, 0–60 s, Pre 1 G_z). A gradual onset rate (GOR) of 0.1 $G_z \cdot s^{-1}$ was employed on the relaxed subject not wearing an anti-G suit from ground level to a base G level of +1.4 G_z (Fig. 2, 60–70 s, duration of 10 s) and from +1.4 G_z to +3 G_z (Fig. 2, 70–90 s, duration of 20 s). The subject was maintained at +3 G_z for 120 s (Fig. 2, 90–220 s) unless the subject requested to abort the trial. Rapid offset rate of 1 $G \cdot s^{-1}$ was used to decelerate the centrifuge from +3 G_z to +1 G_z (Fig. 2, 220–240s, duration of 20 s). The Post centrifuge run measurement lasted for another 60 s as in the Pre segment (Fig. 2, 240–300 s). The maximum, minimum, and mean pupil diameters of the Pre, +3 G_z, and Post sections of the trial were quantified. During the trial, the subject was instructed to remain quiet and passive throughout. In order to maintain mental alertness the subject was also instructed to perform mental arithmetic by subtracting 2's from 1000 (i.e., 998, 996, etc). Furthermore, the subject was reminded to keep his or her eyes open (other than involuntary blinking) throughout the trials. The experimenter and technicians did not communicate with the subjects during the trials.

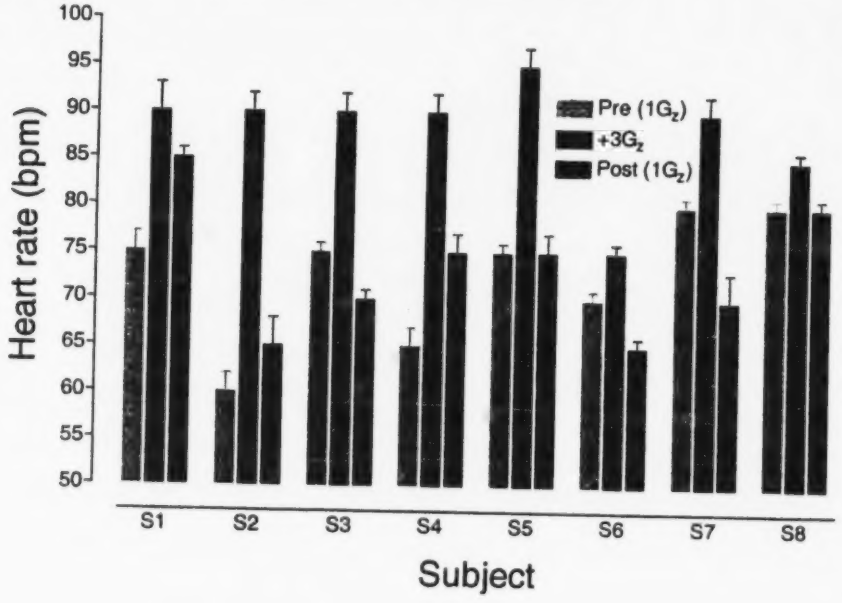

Fig. 1. The mean heart rate of all subjects between Pre (prior to +3 G_z exposure), during +3 G_z, and Post (return to +1 G_z).

Data Collection and Analysis

Pupil diameters from both eyes were recorded on the LTRACK DASI software of the EL-MAR series 2000 systems. The estimate of the pupil center and subsequent pupil size is based on algorithms described by Landau (6). The algorithm for determining the center of the pupil averages the X and Y coordinates of a number of points on the boundary of the pupil and the iris. This information is digitized at 120 Hz with 16-bit resolution. Assuming that the pupil is elliptical, the eye tracking system fits these points with a pre-determined ellipse in such a way that the total mean square error between the ellipse and points that were detected are minimized. Based on this center location, pupil radius is subsequently determined by averaging the distances from the center of the pupil to each of the boundary points. The major axis of the fitted ellipse is used to estimate the pupil diameter. Errors in detecting points on the boundary of the pupil and the fact that the pupil boundary is not perfectly circular can lead to errors in estimating the pupil center and/or the pupil radius. The errors in sampling radii are within 10–20 pixels in individual subject and are repeatable when measured under the exact controlled conditions.

Prior to statistical analysis the pupil diameter data from each eye of each subject was edited off-line using a custom-designed analysis program to remove blinks and involuntary eye movement artifacts which would otherwise skew the data. The mean pupil diameters from the three conditions (Pre, +3 G_z, and Post) were analyzed by repeated measures ANOVA using Statistica by StatSoft. P-values with more than two levels were adjusted using Greenhouse-Geisser's epsilon correction factor.

RESULTS

Six of the subjects experienced visual symptoms such as dimming. Three of these subjects reported a single episode of brief loss of peripheral vision during the first 10 s after reaching +3 G_z and the other three subjects reported a single episode of greyout toward the end of the +3 G_z trials, but none reported blackout. The mean heart rates of each subject during each condition of G exposure are plotted in **Fig. 1**. A one-way ANOVA was performed to compare Pre, +3 G_z, and Post heart rate. Significant differences were found and planned comparison analysis revealed significant heart rate increase, between Pre and +3 G_z ($p = 0.0018$) and between +3 G_z and Post ($p = 0.0008$), but no significant difference was found between Pre and Post conditions. There were no discernable eye movements observed in any subject.

Illustrations of pupil diameter vs. time for each subject during the 5-min centrifuge runs are shown in **Figs. 2 and 3**. Repeated measures ANOVA, three designs (G_z level × two (R-Eye, L-Eye), were performed on the mean pupil size data. There was a significant main effect for G_z level ($F(2,14) = 6.54$, $p = 0.0098$, $\epsilon = 0.9619$) and a significant interaction between G_z level and Eye ($F(2,14) = 5.8366$, $p = 0.0143$). The interaction between G_z level and the R and L eye (illustrated in **Fig. 4**) is due to a lesser difference in pupil diameter between the left and right eye during the Pre section of the centrifuge runs. The main effect for right and left eye was not significant. Close inspection of Fig. 2 and 3 reveals that the pupil diameter in each eye during the sustained +3 G_z segments was significantly smaller than the pupil diameter in each eye during both Pre and Post segments; whereas, the Pre and Post, left and right eye, pupil size did not differ. Planned comparison was performed to confirm these observations (Pre vs. +3 G_z,

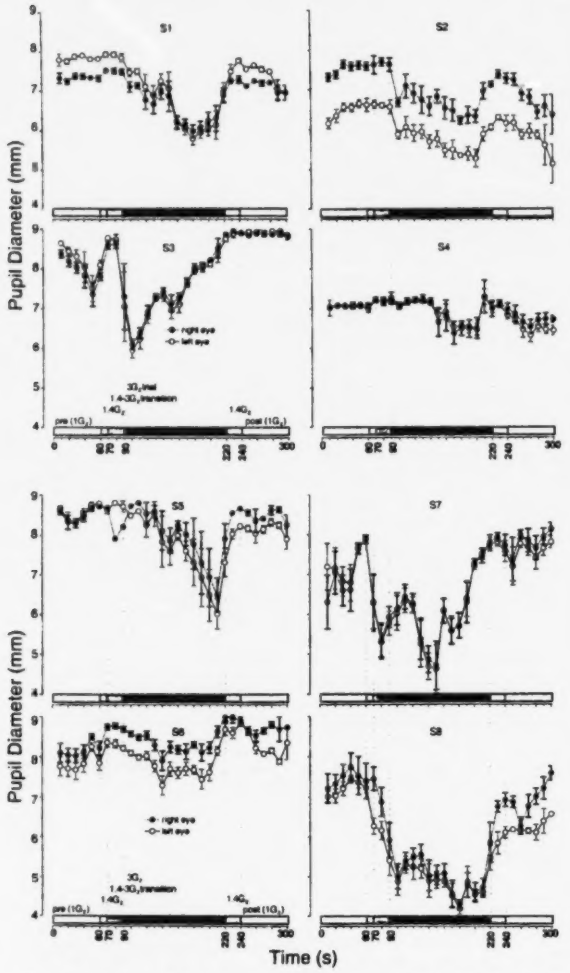

Fig. 2. Separate plots for each subject demonstrate the change in pupil diameter across the experimental profile. Each point on the plots is the mean value of 10 s and the error bars represent standard deviation.

p = 0.0178; +3 G_z vs. Post, p = 0.0236). The percentage decrease of pupil diameter from 1 G_z to +3 G_z of the right and left eyes for each subject is displayed in **Table I.**

The time course of pupil constriction on the onset of G_z varied among individuals. Three of the subjects (S_1, S_3, and S_6) demonstrated significant pupil constriction immediately after they reached +3 G_z. However, three other subjects demonstrated significant pupil constriction during the G transition period before reaching +3 G_z: S_7 during the transition from +1 to +1.4 G_z, S_2 and S_8 during the transition from +1.4 to +3 G_z. Finally, S_5 did not show significant constriction until 40 s after the onset of +3 G_z and S_4 until 60 s into the sustained +3 G_z. Similarly, the time course to reach maximum constriction and the time course to recovery also varied among subjects as illustrated in Figs. 2 and 3.

DISCUSSION

The size of the pupil prior to the presentation of experimental stimulus will limit how much the pupil can constrict or dilate. Therefore, extreme values of pre- and post-stimulus pupillary diameter largely determine the pupillary oscillation. If the pupil is large prior to experimental manipulation, one would not expect a great deal of absolute or percentage dilation, but the potential for constriction of a higher magnitude is present. The average pupil size is 4–6 mm, and the relationship between pre-stimulus values and the magnitude of the response to stimuli is small if pupil diameter is within this range (3). In conjunction with the need to control for vergence eye movements, accommodation, and luminance, the subjects were dark-adapted prior to acceleration in this study. Our findings that pupils constrict rather than dilate during the onset of positive acceleration are in direct contrast to earlier studies (14,1) and they appear to be consistent with the observation that pupils constrict during the hypergravity phase of parabolic flight (9). We attributed the difference essentially to the methods in acquiring and quantifying pupil size.

One difference relates to the lack of control conditions in previous investigation. The size of the pupil can be influenced by many environmental, physiological, and psychological factors. For example, variations in ambient illumination, and changes to accommodation can affect pupil size. The diameter of the pupil decreases approximately as the logarithm of retinal illuminance increases (10). Conversely, the diameter of the pupil is reduced approximately as the inverse of target distance (13). Pupil size also changes with different physiological states such as fatigue, eye movements, intraocular pressure as well as stimulation of other sensory organs such as hearing and smell (12). It is also influenced by changes in the subject's emotional and mental states. For example, surprise, fear, pain, and degree of difficulty in mental task cause the pupil to dilate (4). In the present study the emotional factors were reduced, as the subjects were experienced. The second difference relates to the method in data acqui-

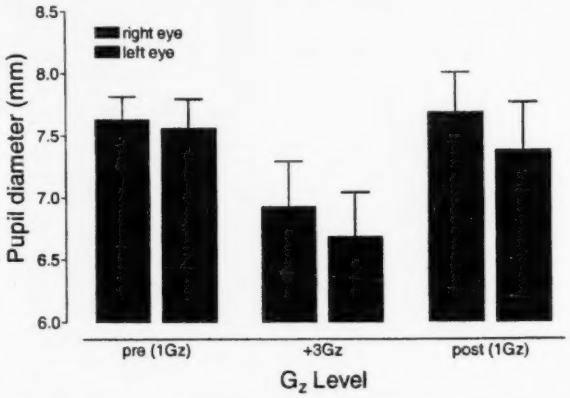

Fig. 3. The interaction of mean pupil diameter against G level for the right and left eye (error bars represent standard error of means).

sition and analysis of the pupil size. Previous investigations relied on measurement from still photographs and filmstrips. The typical sampling rate of these techniques is very low. The wide range of responses and the spontaneous fluctuation of tone and activity of the system suggest that high sampling rate and highly sensitive technique to detect the pupil size would be more informative.

Furthermore, previous investigations relied heavily on descriptive observation, there were no quantitative data presented to substantiate the claim. Other studies using flash photography could cause a light reflex that is considered to be the most important pupillary reaction that affects the pupil diameter. It has been reported that the light reflex is the primary determinant of pupil size. Therefore it is important to keep the amount of light impinging on the retinas as constant as possible. The luminance of a stimulus target on which the subject's gaze falls must be uniform. As the subjects alter their fixation point or when they are exposed to sudden flash of light, different brightness levels exist producing changes in pupil size (2,7). In contrast, this study provides real time measurements under tightly controlled condition of emotional and mental state, illumination level, and accommodation.

In this study, the time course of pupil constriction can roughly divide the subjects into two groups: a rapid constriction of the pupil during transition to +3 G_z and a more gradual constriction of the pupil during the same transition. However, the time course of pupil constriction appears not to be related to the visual symptoms that subjects experienced (as reported in the Results section). The pupils of healthy, well-rested subjects in darkness usually are quite stable over minutes at a time. They remain large and steady as long as the subject stays fully alert; however, under the influence of boredom or fatigue, the pupils may begin to oscillate. This might account for the oscillation observed in S_7 and S_8 prior to +G_z exposure. A steadily declining pupil size is common when subjects remain quietly in darkness without anything to do. The steady decline in pupil diameter during the controlled period of S_3 might have been attributed to the fatigue waves. Although the time course of pupillary diameter changes in the left and right pupil of S_2 during +G_z exposure is consistent, there is a significant difference in the size of the left and right pupil. This observation was consistent with repeated measurements 1 wk apart, which suggested that there might be an inherent neuroanatomical difference.

It was hypothesized that the increase in the size of the pupil was a response of the sympathetic nervous system to the onset of a stressful increased acceleration and to cerebral hypoxia (1). Using a cervical pressure cuff Rossen et al. (11) reported that moderate dilatation of the pupils was observed 12 to 20 s after beginning of cerebral circulation arrest. However, other clinical studies revealed that patients with different degrees of respiratory insufficiency demonstrated pupil constriction (4). Dilatation of the pupil was not observed even when anoxia was severe. These clinical findings were further substantiated by exposing rabbits to obstructive anoxia, a pronounced constriction of the pupils was demonstrated until death and the pupil dilated only about the time of circulatory arrest. Therefore, the reliability of anoxia as a cause for pupil dilatation is doubtful. On the other hand, our results are consistent with the study by Oosterveld (9) who reported that three subjects showed pupil constriction when exposed to +2.8 G_z in the human centrifuge. Their pupils dilated when the accelerative force was returned to 1G. Results from the current and the previous (9) centrifuge studies are partially supported by the findings of the parabolic flight studies (briefly mentioned in the Introduction) in which microgravity and hypergravity induced pupil dilation and constriction respectively. However, it should be noted that during parabolic flight maneuvers, each microgravity phase was preceded by a hypergravity period. Therefore, the effects of +G_z transition on the vestibular and cardiovascular systems might have influenced the subjects' response. In addition, there was no information provided regarding the time course of pupil response within the microgravity and hypergravity phase. For example, did the pupil remain dilated throughout the microgravity phase and remain constricted during the hypergravity phase?

There is a constant modulation of pupillary responses brought about by the spontaneous shifts in autonomic innervation. The dilator papillae serve to retract the iris and enlarge the pupil and are innervated mostly by the sympathetic nerve fibers. The sphincter pupillae serve to close the pupil by expanding the iris. The point of origin for control of the sphincter pupillae is a parasympathetic action through fibers originating in the Edinger-Westphal's nucleus in the mesencephalon, which receives direct otolith fibers. The neural pathway continues with efferent fibers exiting the brain through the third cranial nerve leading to the eye, and is then interrupted by a synapse at the ciliary ganglion where excitatory projections are made to the sphincter pupillae. Changes in pupil size and heart rate are completely abolished following vestibular lesion (8). It appears that the reflex regulation of pupil size under acceleration is attributed more to fluctuations in the discharge through the ciliary ganglion than to the sympathetic innervation. The functional significance of this reflex constriction under acceleration is unclear and requires further investigation. A small pupil has long been known to help vision by enhancing the depth of focus of the eye. Perhaps the reflex constriction is to help maintain vision during increased acceleration when visual symptoms such as greyout and loss of peripheral vision develop.

ACKNOWLEDGMENTS

Thanks are due to Mr. Jamie Joseph, Ms. Heather Devine, and Mr. Jim Malone for their technical assistance in data collection and analysis.

REFERENCES

1. Duane TD, Beckman EL, Coburn KR. Limitation of ocular motility and pupillary dilatation in human beings during positive acceleration. Invest Ophthalmol 1962; 1:136–41.

2. Goldwater BC. Psychological significance of pupillary movements. Psychol Bull 1972; 77:340–55.
3. Hansmann D, Semmlow J, Stark L. A physiological basis of pupillary dynamics. In: Janisse MO, ed. Pupillary dynamics and behaviour. New York: Plenum Press; 1974.
4. Jordanov J, Ruben H. Reliability of pupillary changes as a clinical sign of hypoxia. Lancet 1967 (Oct); 28:915–7.
5. Kahneman D. Pupillary, heart rate, and skin resistance changes during a mental task. J Exp Psychol 1969; 79:1, 164–7.
6. Landau UM. Estimation of a circular arc centre and its radius. Computer Vision, Graphics and Image Processing 1987; 28: 317–26.
7. Loewenfeld IE. Comment on Hess's finding. Surv Ophthalmol 1966; 11:291–4.
8. Morrsion AR, Pompeiano O. Vestibular influence during sleep. VI. Vestibular control of autonomic functions during the rapid eye movements of desynchronized sleep. Arch Ital Biol 1970; 108:154–80.
9. Oosterveld WJ, de Jong HAA, Smit J, Kortschot HW. Pupil size and microgravity. Adv Otorhinolaryngol 1988; 42:9–12.
10. Reeves P. The response of the average pupil to various intensities of light. J Opt Soc Am 1920; 4:35–43.
11. Rossen R, Kabat H, Anderson JP. Acute arrest of cerebral circulation in man. Arch Neurol Psych 1943; 50:510–28.
12. Rumpf G. Pupillary reaction while smelling and hearing. Proceedings of the Congress of Orbital Diseases. Amsterdam; 1973.
13. Semmlow J, Stark L. Pupil movements to light and accommodative stimulation: a comparative study. Vision Res 1971; 13: 1087–100.
14. Wilson RC, Canfield AA. The effects of increased positive radial acceleration upon pupillary response. In: Warren MD, et al. Psychological research on the human centrifuge: final report. Los Angeles: Department of Psychology, University of Southern California; June 1951. Tech. Rept N6-ori-77, Task order 3.